PATENT
Docket No. 529492000100

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Mark A. SCHENA

Serial No.: 09/613,006

Filing Date: July 10, 2000

For: MICROARRAY METHOD OF GENOTYPING MULTIPLE SAMPLES AT MULTIPLE LOCI

Examiner: B. Forman

Group Art Unit: 1634

DECLARATION OF MARK A. SCHENA PURSUANT TO 37 C.F.R § 1.131

Commissioner for Patents
Alexandria, VA 22313-1450

Dear Sir:

I, Mark A. Schena, declare as follows:

1. I am the sole inventor named in the above-referenced patent application, and I am familiar with the contents thereof.

2. The work was completed by me or under my direction.

3. I conceived of the invention claimed in the subject application prior to February 16, 2000.

4. I have worked diligently on reducing to practice the claimed invention in the subject application since before February 16, 2000 until the application was filed on July 10, 2000.

5. The following paragraph summarizes the document attached to this declaration which is submitted as evidence that I conceived of the claimed invention in the subject application prior to February 16, 2000. The attached document was prepared in the U.S. All of the activities reported in the document occurred in the U.S. With respect to this document, dates and portions that are not relevant to this declaration have been redacted.

6. Exhibit A is evidence of my conception of the claimed invention in the subject application. The conception was made on a date prior to February 16, 2000.

7. The following paragraph summarizes the documents attached to this declaration which are submitted as evidence that I was diligent in reducing the claimed invention in the subject application to practice. All of the attached documents were prepared in the U.S. prior to the filing date of July 10, 2000. All of the activities reported in these documents occurred in the U.S. in a diligent manner during a period commencing prior to February 16, 2000 and ending prior to July 10, 2000. With respect to all of these documents, dates (all of which are prior to July 10, 2000) and portions that are not relevant to this declaration have been redacted.

8. Exhibit B shows computer files and pages from my laboratory notebooks that show that I worked diligently on the reduction of the claimed invention to practice. Pages 1 – 6 show computer files of data generated from experiments. Page 7 shows the sequences of oligonucleotides used in experiments. Page 8 shows a protocol used in the experiments. Page 9 shows the sequences of oligonucleotides used in the experiments. Pages 10 – 11 show a sequence alignment generated in the experiments. Pages 12 – 14 show the sequences of oligonucleotides used in the experiments. Pages 15 – 18 show nucleotide sequences analyzed in the experiments. Pages 19 – 21 show protocols used in experiments. Page 22 shows numerical microarray data. Pages 23 – 30 show pictorial microarray data.

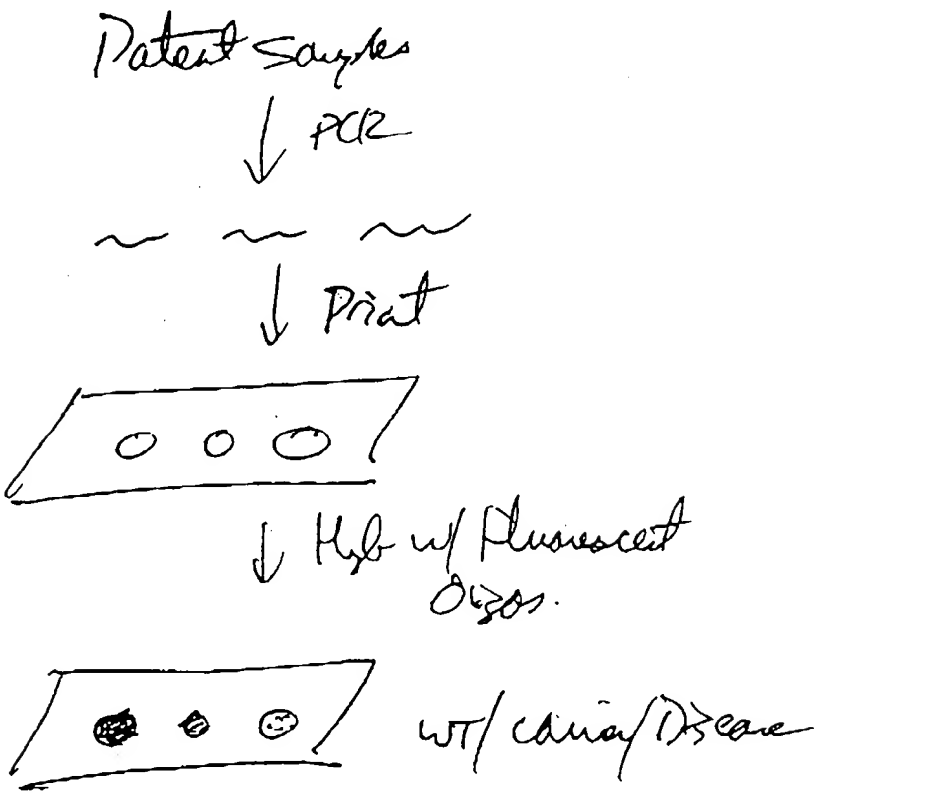
I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

5/3/03
Date

Mark A. Schena

Exhibit A

NOTES



∴ Multiple patients + multiple diseases in one test ⇒ Cost-effective!

- Visit booth
- Sharon re: Stratagene MS?

Exhibit B

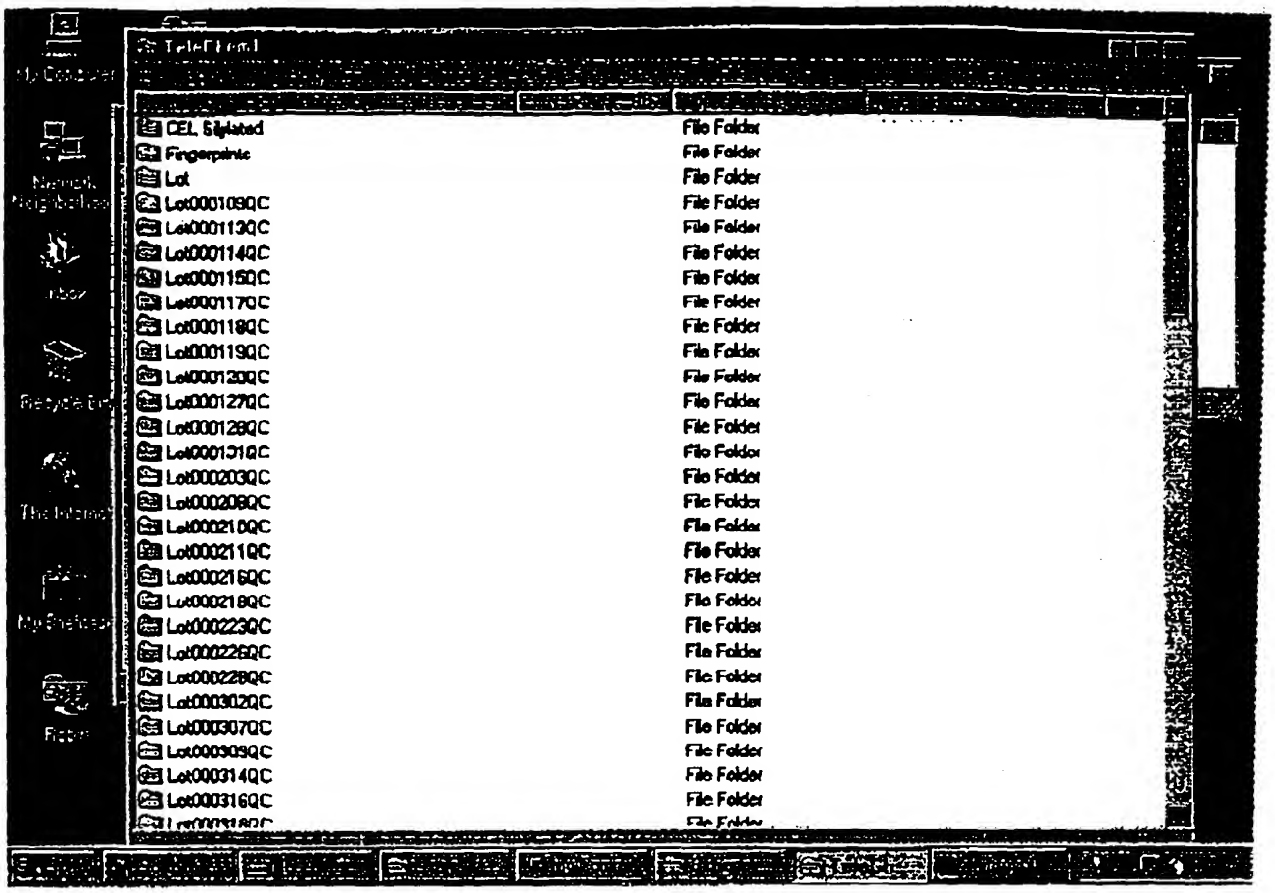

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Exhibit B

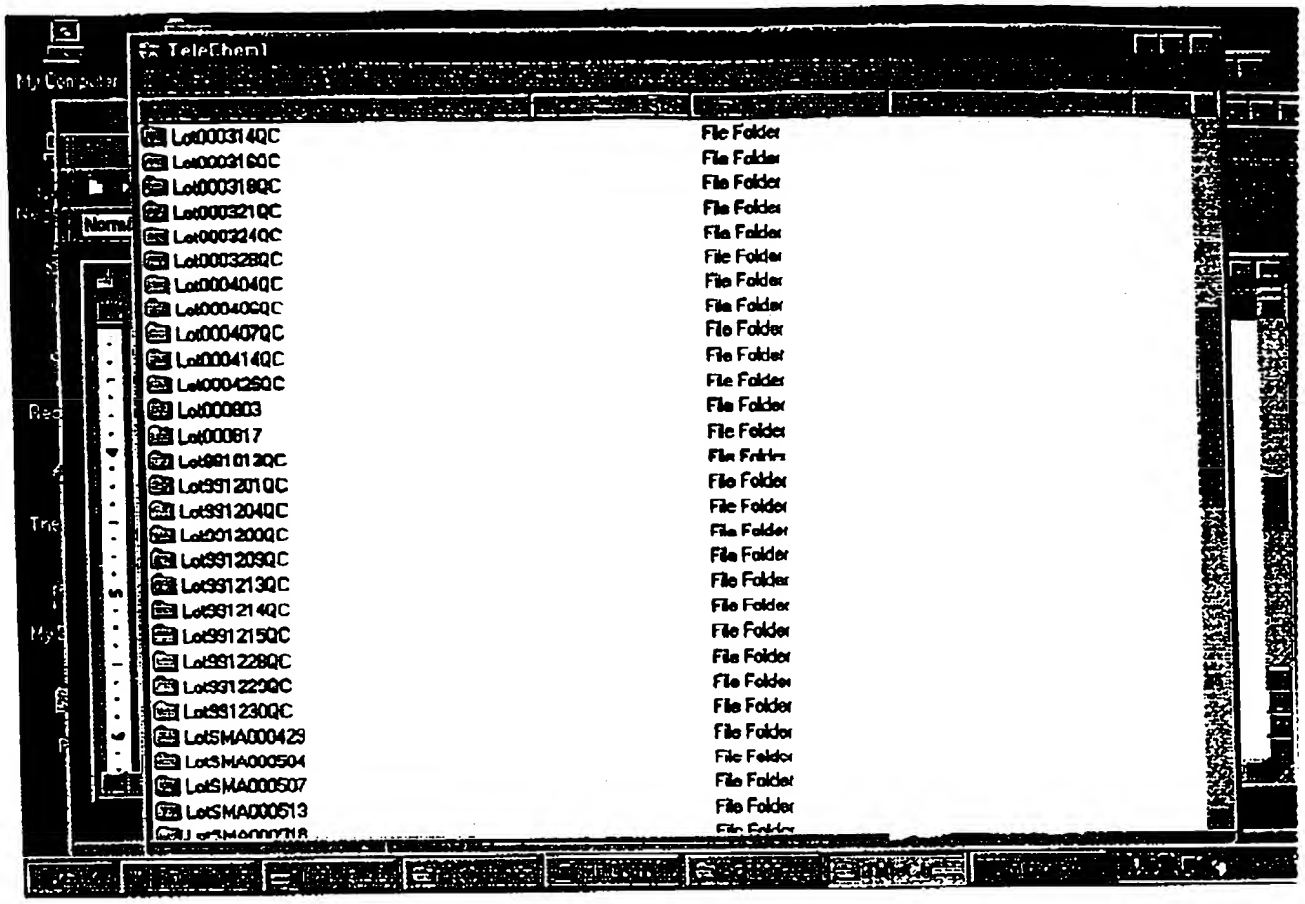
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Exhibit B

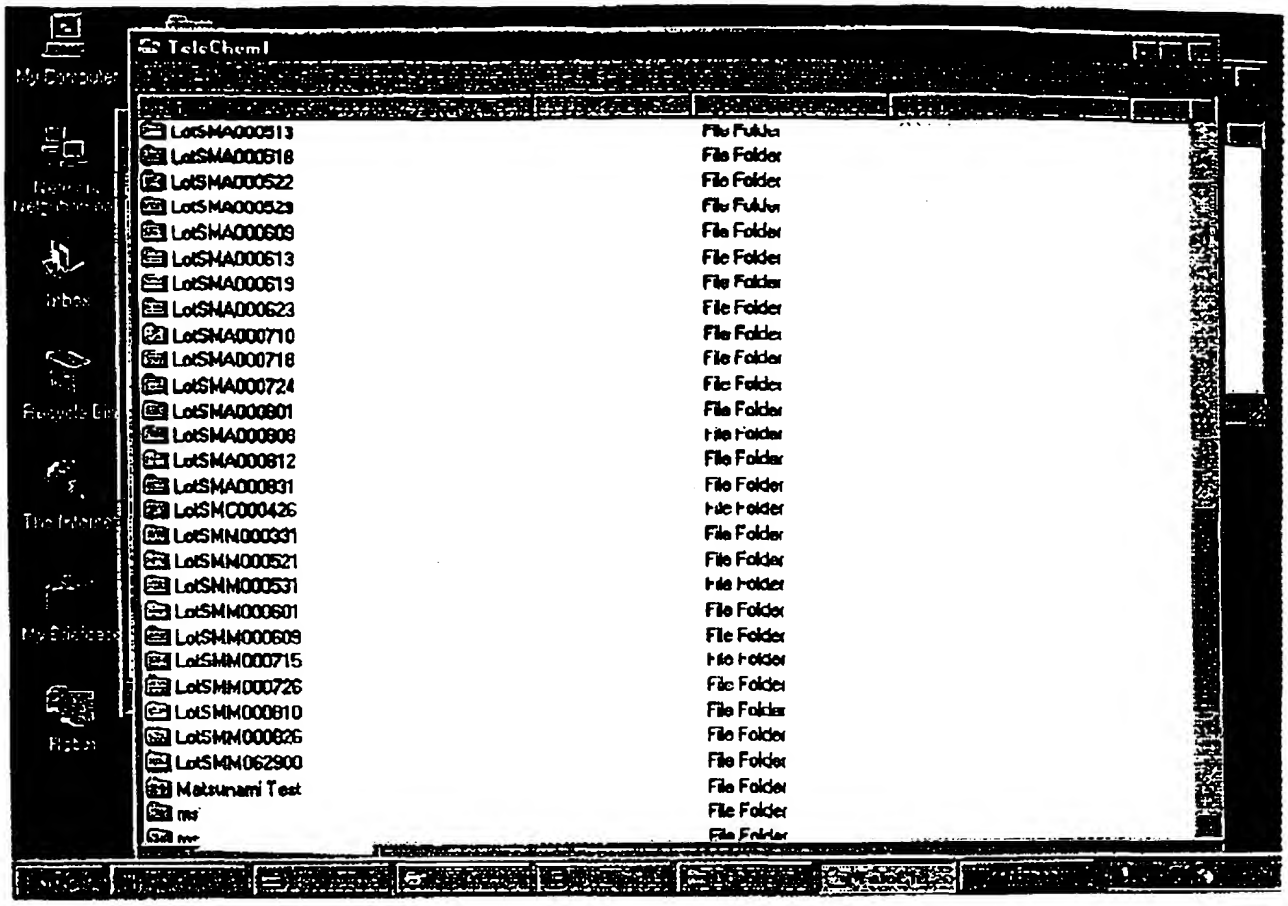
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Exhibit B

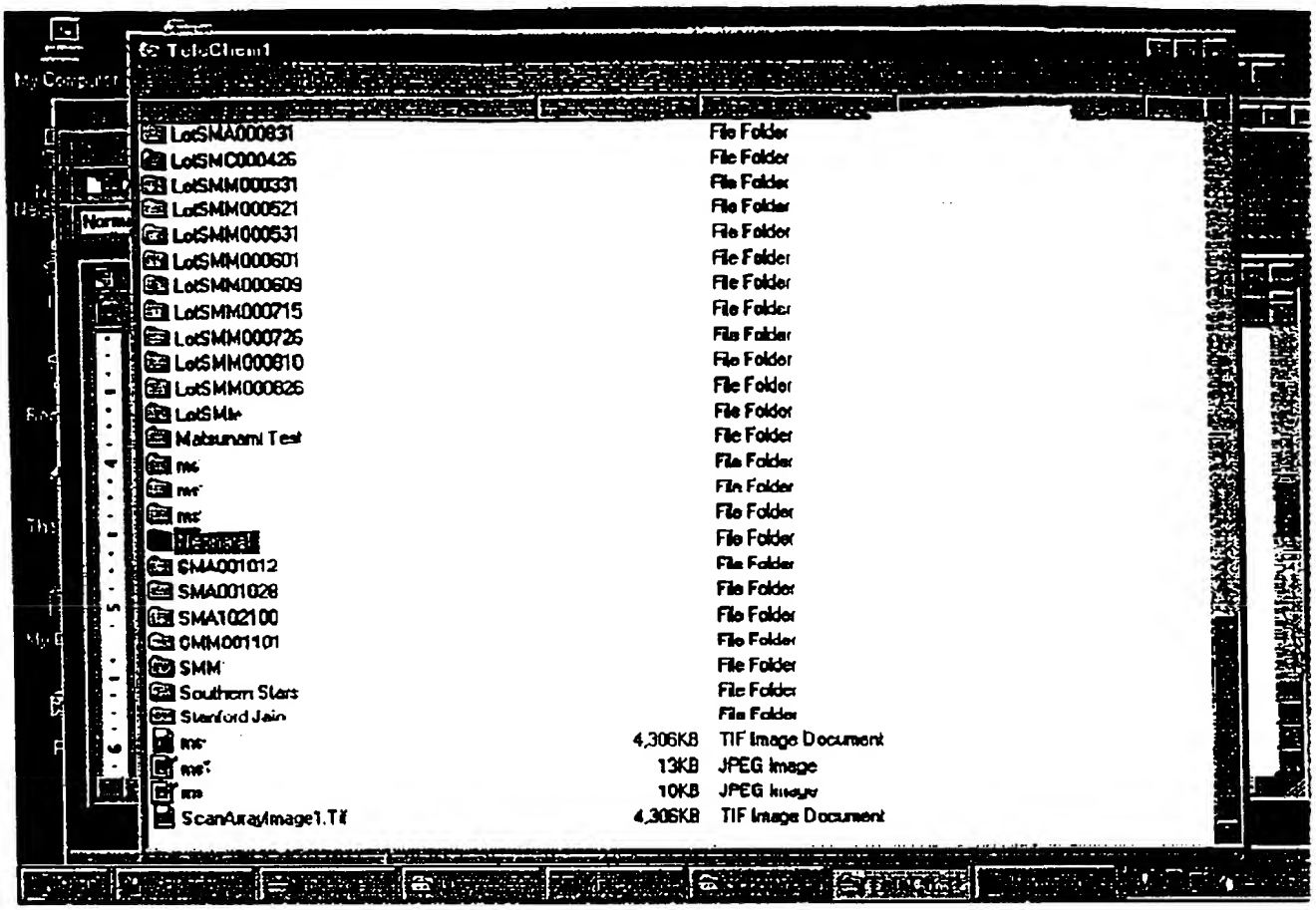
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Exhibit B

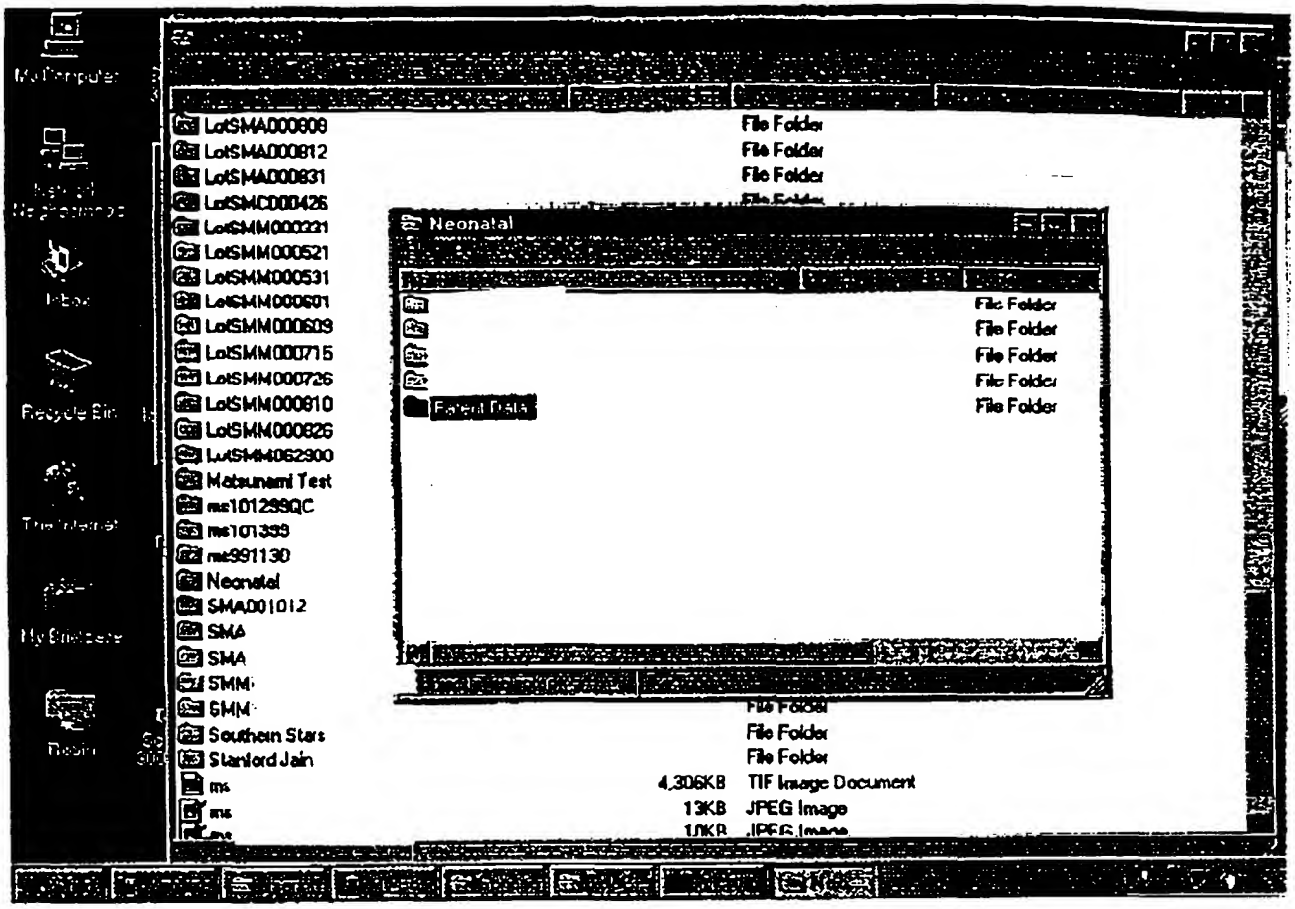
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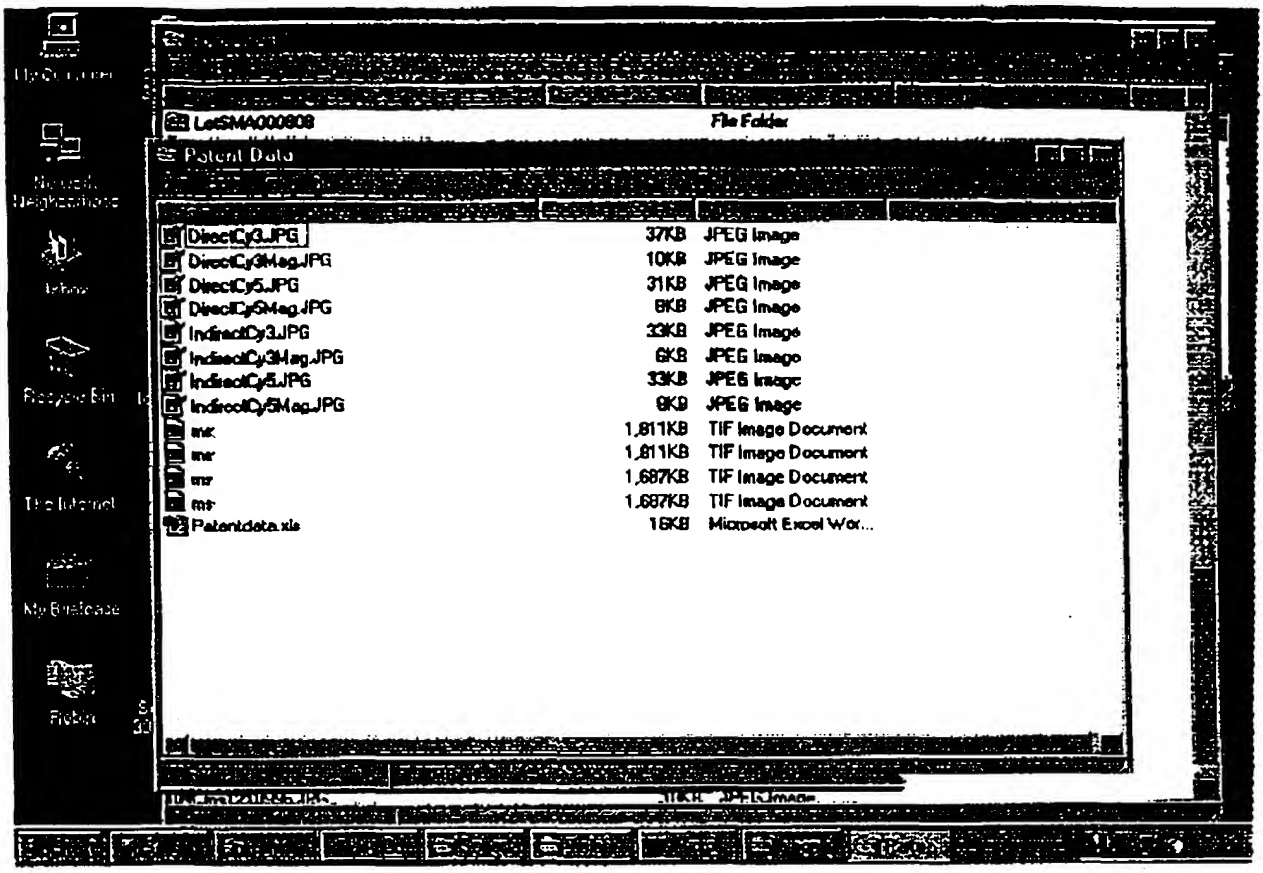
Exhibit B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1,811KB TIF Image Document
1,687KB TIF Image Document
1,687KB TIF Image Document
Patentdata.xls 16KB Microsoft Excel Wor...

Exhibit B

ARDC-110 (Green Label, Sickle Cell WT)
5' NGA CTC CTG (A/T)GG AGA A 3'
N = Cy3

A1 → E1
+
A3 → E3

ARDC-111 (Red Label, Sickle Cell C allele)
5' NGA CTC CTA (A/T)GG AGA A 3'
N = Cy5

ARDC-112 (Red Label, Sickle Cell WT)
5' NTG GTG GTG AGG CCC T 3'
N = Cy5

ARDC-113 (Green Label, Sickle Cell S allele)
5' NTG GTG GTA AGG CCC T 3'
N – Cy3

ARDC-114 (Green Label, CF WT)
5' NAT CAT CTT TGG TGT T 3'
N = Cy3

ARDC-115 (Red Label, CF ΔF508)
5' NTA TCA TCG GTG TTT C 3'
N = Cy5

ARDC-116 (Red Label, GALT Q188R WT)
5' NCA CTG CCA GGT AAG G 3'
N = Cy5

ARDC-117 (Green Label, GALT Q188R mutant)
5' NCA CTG CCG GGT AAG G 3'
N = Cy3

ARDC-118 (Green Label, N314D WT)
5' NCA ACT GGA ACC ATT G 3'
N = Cy3

ARDC-119 (Red Label, N314D mutant)
5' NCA ACT GGG ACC ATT G 3'
N = Cy5

b. Plasmid DNA can be prepared by alkaline lysis and purified. The 96-well REAL prep (Qiagen #SQ811 and #19504) facilitates rapid preparation.

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Protocol 4. Microarray manufacture and processing.

Reagents and Equipment

- Micro-spotting robot (Various)
- Stealth Micro Spotting Device (TeleChem)
- SuperAldehyde Substrates (TeleChem)

Method

1. Obtain silylated (active aldehyde) microscope slides (CEL Associates).
2. Print amino-linked cDNAs using a micro-spotting device according the to manufacturer's instructions.
3. Allow printed microarrays to dry overnight in a slide box[a].
4. Soak slides twice in 0.2% SDS for 2 min at room temperature with vigorous agitation[b].
5. Soak slides twice in ddH2O for 2 min at room temperature with vigorous agitation.
6. Transfer slides into ddH2O at 95-100°C for 2 min to allow DNA denaturation.
7. Allow slides to dry thoroughly at room temperature (~5 min).
8. Transfer slides into a sodium borohydride solution[c] for 5 min at room temperature to reduce free aldehydes.
9. Rinse slides three times in 0.2% SDS for 1 min each at room temperature.
10. Rinse slides once in ddH2O for 1 min at room temperature.
11. Submerge slides in ddH2O at 95-100°C for 2 seconds[d].
12. Allow the slides to air dry and store in the dark at 25°C (stable for >1 year).

a. Drying increases crosslinking efficiency. Several days or more is acceptable.

b. This step removes salt and unbound DNA.

c. Dissolve 1.0 g $NaBH_4$ in 300 ml phosphate buffered saline (PBS). Add 100 ml 100% ethanol to reduce bubbling. Prepare JUST PRIOR to use!

d. Heating the slides greatly aids in the drying process.

Method

1. Prepare a 15-mer[a] oligonucleotide microarray wherein the central (8[th]) position identifies the polymorphism or mutation in the fluorescent sample[b]. Microarrays are made by spotting 10-100 pmole/µl oligonucleotide in 1X micro-spotting solution.
2. Process the microarray to remove unbound 15-mer.
3. Prepare a fluorescent sample by PCR amplification of the locus encompassing the polymorphism or mutation[c]. Use ~1/10 of a 100 µl PCR reaction for hybridization of a sample that contains <1,000 loci. Purify the sample prior to hybridization by ethanol precipitation or spin column purification.
4. Denature the sample by boiling for 2 min prior to hybridization[d].
5. Hybridize the fluorescent sample to the oligonucleotide microarray for 4 hrs at 42°C[e].
6. Wash the microarray to remove unhybridized sample as follows: twice for 5 min each at room temperature in 2X SSC, once for 5 min at room temperature in 2X SSC.
7. Allow the microarray to air dry.
8. Scan the microarray at the highest PMT and laser settings that preserve linearity and minimize background[f].
9. Quantitate fluorescent intensities with ImaGene software.

a. Oligonucleotides must be coupled covalently to the solid support. We have used microscope slides with reactive aldehyde groups and primary amines on the oligonucleotides to mediate covalent end attachment.

b. The central or 8[th] position in a 15-mer is used to identify a single base polymorphism or mutation by hybridization. For a marker in which the wild type is a "G" and the mutant is a "T", the two complementary 15-mers would be identical except at position 8 in which the wild type 15-mer would contain a "C" and the mutant oligonucleotide would contain an "A".

c. Fluorescent primers spanning the allele of interest by ~60-bp will yield a product that hybridizes efficiently to the oligonucleotide microarray.

d. Double-stranded fluorescent products must be denatured prior to hybridization. Single-stranded fluorescent samples made by linear PCR are preferable.

e. Hybridization temperature should be ~10°C below the T_m. 42°C works well for 15-mers. The temperature should be adjusted for longer or shorter oligonucleotides.

f. On the ScanArray 3000, laser and PMT settings of 70% and 80%, respectively, work well for most genotyping applications.

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Literature Cited

1. M. Schena and R.W. Davis (1998). Genes, Genomes and Chips. In DNA Microarrays: A Practical Approach (ed. M. Schena), Oxford University Press, Oxford, UK, in press.
2. Schena, M. and R.W. Davis (1998). Parallel Analysis with Biological Chips. In PCR Methods Manual (eds. M. Innis, D. Gelfand, J. Sninsky), Academic Press, San Diego, in press.
3. Lemieux, B., Aharoni, A., and M. Schena (1998). Overview of DNA Chip Technology. Molecular Breeding 4, 277-289.
4. Schena, M., Heller, R.A., Theriault, T.P., Konrad, K., Lachenmeier, E., and R.W. Davis (1998). Microarrays: biotechnology's discovery platform for functional genomics. Trends in Biotechnology 16, 301-306.
5. Heller, R.A., Schena, M., Chai, A., Shalon, D., Bedilion, T., Gilmore, J., Woolley, D.E., and Davis, R.W. (1997). Discovery and analysis of inflammatory disease-related genes using cDNA microarrays. Proceedings of the National Academy of Sciences USA 94, 2150-2155.
6. Schena, M., Shalon, D., Heller, R., Chai, A., Brown, P.O., and R.W. Davis. (1996). Parallel Human Genome Analysis: Microarray-Based Expression Monitoring of 1,000 Genes. Proceedings of the National Academy of Sciences USA 93, 10614-10619.
7. Schena, M. (1996). Genome analysis with gene expression microarrays. BioEssays 18, 427-431.
8. Schena, M., Shalon, D., Davis, R.W. and Brown, P.O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270, 467-470.

Special thanks to Mark Schena and Ronald W. Davis, Department of Biochemistry, Beckman Center, Stanford University, for contributions to these

Exhibit B

EOS BIOTECHNOLOGY: OLIGO RECORD FILE

ArrayIt
Run Title: 340 Plate 1
NOTES: "Cy3/Cy5/Aminolink 15mers, and Aminolink 20mers"

SERIAL NUMBER: 300003401

#	NAME 5'SP	5' --> 3' SEQUENCE	Plate Row	Plate Col.	Run Comment
	amount (ug)	amount (nmol)			
1	ARDC-110 CY3	GACTCCTGWGGAGAA	A	1	23.42 4.82
2	ARDC-113 CY3	TGGTGGTAAGGCCCT	B	1	32.92 6.77
3	ARDC-114 CY3	ATCATCTTTGGTGTT	C	1	33.30 6.93
4	ARDC-117 CY3	CACTGCCGGGTAAGC	D	1	29.24 6.02
5	ARDC-118 CY3	CAACTGGAACCATTG	E	1	25.85 5.39
6	ARDC-110 CY3	GACTCCTGWGGAGAA	F	1	24.54 5.05
7	ARDC-113 CY3	TGGTGGTAAGGCCCT	G	1	29.27 6.02
8	ARDC-114 CY3	ATCATCTTTGGTGTT	H	1	27.18 5.66
9	ARDC-117 CY3	CACTGCCGGGTAAGG	A	2	26.59 5.48
10	ARDC-118 CY3	CAACTGGAACCATTG	B	2	25.93 5.40
11	ARDC-110 CY3	GACTCCTGWGGAGAA	C	2	21.59 4.44
12	ARDC-113 CY3	TGGTGGTAAGGCCCT	D	2	34.51 7.10
13	ARDC-114 CY3	ATCATCTTTGGTGTT	E	2	36.17 7.53
14	ARDC-117 CY3	CACTGCCGGGTAAGG	F	2	28.14 5.80
15	ARDC-118 CY3	CAACTGGAACCATTG	G	2	27.07 5.64
16		H 2			
17	ARDC-111 CY5	GACTCCTAWGGAGAA	A	3	26.60 5.49
18	ARDC-112 CY5	TGGTGGTGAGGCCCT	B	3	32.80 6.72
19	ARDC-115 CY5	TATCATCGGTGTTTC	C	3	20.76 4.34
20	ARDC-116 CY5	CACTGCCAGGTAAGG	D	3	23.47 4.85
21	ARDC-119 CY5	CAACTGGGACCATTG	E	3	22.62 4.70
22	ARDC-111 CY5	GACTCCTAWGGAGAA	F	3	22.88 4.72
23	ARDC-112 CY5	TGGTGGTGAGGCCCT	G	3	26.28 5.39
24	ARDC-115 CY5	TATCATCGGTGTTTC	H	3	17.87 3.73
25	ARDC-116 CY5	CACTGCCAGGTAAGG	A	4	22.40 4.63
26	ARDC-119 CY5	CAACTGGGACCATTG	B	4	31.33 6.51
27	ARDC-111 CY5	GACTCCTAWGGAGAA	C	4	24.06 4.97
28	ARDC-112 CY5	TGGTGGTGAGGCCCT	D	4	36.45 7.47
29	ARDC-115 CY5	TATCATCGGTGTTTC	E	4	23.95 5.00
30	ARDC-116 CY5	CACTGCCAGGTAAGG	F	4	26.93 5.56
31	ARDC-119 CY5	CAACTGGGACCATTG	G	4	31.42 6.52
32		H 4			
33	ARDC-120 L	TTCTCCWCAGGAGTC	A	5	21.64 4.38
34	ARDC-121 L	AGGGCCTCACCACCA	B	5	40.17 8.16
35	ARDC-122 L	AACACCAAAGATGAT	C	5	30.43 6.09
36	ARDC-123 L	CCTTACCTGGCAGTG	D	5	26.44 5.33
37	ARDC-124 L	CAATGGTTCCAGTTG	E	5	28.09 5.62
38	ARDC-120 L	TTCTCCWCAGGAGTC	F	5	20.54 4.16
39	ARDC-121 L	AGGGCCTCACCACCA	G	5	21.05 4.28
40	ARDC-122 L	AACACCAAAGATGAT	H	5	22.33 4.47
41	ARDC-123 L	CCTTACCTGGCAGTG	A	6	27.25 5.49
42	ARDC-124 L	CAATGGTTCCAGTTG	B	6	31.92 6.38
43	ARDC-120 L	TTCTCCWCAGGAGTC	C	6	19.84 4.02
44	ARDC-121 L	AGGGCCTCACCACCA	D	6	22.79 4.63
45	ARDC-122 L	AACACCAAAGATGAT	E	6	30.10 6.03
46	ARDC-123 L	CCTTACCTGGCAGTG	F	6	29.27 5.90
47	ARDC-124 L	CAATGGTTCCAGTTG	G	6	25.90 5.18

Cy3 probe

Cy5 probe

Assume 5 want us

Control Oligos.

Exhibit B

gb|AF186609.1|AF186609	Homo sapiens haplotype A7 beta-globi...	1744	0.0	
gb|AF186608.1|AF186608	Homo sapiens haplotype A11 beta-glob...	1744	0.0	
gb|AF186606.1|AF186606	Homo sapiens haplotype A1 beta-globi...	1744	0.0	
gb|AF186620.1|AF186620	Homo sapiens haplotype D6 beta-globi...	1737	0.0	
gb|AF186615.1|AF186615	Homo sapiens haplotype B18 beta-glob...	1737	0.0	
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gb|AF186619.1|AF186619	Homo sapiens haplotype D4 beta-globi...	1729	0.0	
gb|AF186618.1|AF186618	Homo sapiens haplotype C2 beta-globi...	1729	0.0	
gb|AF186614.1|AF186614	Homo sapiens haplotype B22 beta-glob...	1729	0.0	
gb|AF186617.1|AF186617	Homo sapiens haplotype C19 beta-glob...	1721	0.0	
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gb|AF007546.1|AF007546	Homo sapiens beta-globin gene, compl...	1461	0.0	
emb|V00499.1|HSBGL1	Human germ line gene for beta-globin	1441	0.0	
gb|U20221.1|HSU20221	Human thalassemia beta globin gene, co...	1356	0.0	
gb|AF083884.1|AF083884	Homo sapiens mutant beta-globin (HBB...	1174	0.0	
gb|L00330.1|COLHBBSCP	monkey (c.polykomos) beta-globin gene...	1136	0.0	
gb|K01859.1|HUMHBMYN	Human beta globin Miyada gene on chromo...	1025	0.0	
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emb|V00505.1|HSDGL1	Human gene for delta-globin	706	0.0	
gb|M21835.1|GGAAMBD	Orangutan delta globin gene, complete cds	680	0.0	
gb|M19061.1|AMKMAD	Spider monkey (A.geoffroyi) delta-globin...	614	e-179	
gb|M25660.1|HUMHBRBPN	Human beta-hemoglobin gene, exons 1 an...	597	e-168	
emb|X00623.1|HSGLOB	Human beta-globin gene alternative tran...	595	e-167	
gb|AF105971.1|AF105971	Homo sapiens beta globin (HBB) gene,...	579	e-163	
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gb|J04429.1|TARHBB	T.syrichta beta globin gene, complete cds	575	e-161	
gb|U60902.1|OCU60902	Otolemur crassicaudatus epsilon-, gamm...	484	e-134	
gb|M61740.1|GCRDELGLB	G.crassicaudatus beta globin gene, co...	484	e-134	
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gb|AF117710.1|AF117710	Homo sapiens hemoglobin beta chain (...	438	e-120	
emb|V00500.1|MSBOTLX	Human messenger RNA for beta-globin	438	e-120	
emb|V00497.1|HSTGL1	Human messenger RNA for beta-globin	438	e-120	
gb|M25113.1|HUMHBEMOR	Human sickle beta-hemoglobin mRNA	438	e-120	
gb|M25661.1|HUMHBDPN	Human delta-hemoglobin gene, exons 1 a...	385	e-104	
gb|M15734.1|LEMHBR	Lemur (brown) beta-globin gene, complete...	378	e-102	
gb|J00335.1|COLHBDPSC	Monkey (colobus) delta-globin pseudog...	373	e-101	
gb|J00332.1|RAMHBDPS	monkey (aqubig) silent delta-globin gene	365	2e-98	
ref|NM_000519.2||	Homo sapiens hemoglobin, delta (HBD), mRNA	363	8e-98	
gb|M25079.1|HUMHBTYGLA	Human sickle cell beta-globin mRNA, c...	359	1e-96	
gb|J00334.1|MACHBDPS1	Monkey (rhesus) delta-globin pseudoge...	333	7e-89	
emb|X00347.1|RBMHBAN	Lepus europaeus adult beta-globin gene	281	8e-74	
gb|J04428.1|TARHBD	T.syrichta delta-globin gene, complete cds	281	2e-73	
emb|V00878.1|OCBGL1	Rabbit gene for beta-globin	276	1e-71	
gb|K03256.1|RABHBBR1B1	Rabbit beta1-globin gene (allele 2),...	276	1e-71	
gb|J00660.1|RABHBBAB	Rabbit beta1-globin with 2 ivs, type 2...	276	1e-71	
gb|M18818.1|RABBCLOB	Rabbit beta-like globin gene cluster e...	276	1e-71	
emb|V00882.1|OCBCLO	Rabbit (O. cuniculus) gene for beta-globin	268	3e-69	
gb|J00658.1|RABHBBAA	rabbit beta1-globin with 2 ivs, type 1...	268	3e-69	
emb|V00879.1|OCBGL2	Messenger RNA for rabbit beta-globin	256	1e-65	
gb|M61453.1|BOVBG	Bovine Beta globin gene and globin (PEI-1...	238	1e-60	
emb|X00376.1|BTCLG2	Bovine adult beta-globin gene	238	3e-60	
emb|X86781.1|SSBETAC	S.scrofa beta-globin gene	222	2e-55	
emb|X14728.1|OARCGLOB	Sheep beta-C globin gene	222	2e-55	
gb|M15388.1|GOTHBGA	Goat gamma-globin gene, complete cds	222	2e-55	
emb|X14727.1|OARBGLOB	Sheep beta-B globin gene	214	5e-53	
gb|M15389.1|GOTHBAB	Goat cysteine-beta-globin gene, complet...	202	2e-49	
gb|M15387.1|GOTHBBAA	Goat alanine-beta-globin gene, complet...	202	2e-49	
gb|U01378.1|U01378	Ovis aries fetal globin gene, complete cds	186	1e-44	

gb|U01317.1|HUMHBB Human beta globin region on chromosome 11
Length = 73308

Score = 2260 bits (1140), Expect = 0.0
Identities = 1140/1140 (100%)
Strand = Plus / Plus

```
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Sbjct: 61621  cttaccagaaaggttttaatccaaatcaaggagaagatatgcttagaactgaggtagagttt 61680

Query: 61     tcatccattctgtcctgtaagtattttgcatattctggagacgcaggaagagatccatct 120
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Sbjct: 61681  tcatccattctgtcctgtaagtattttgcatattctggagacgcaggaagagatccatct 61740

Query: 121    acatatcccaaagctgaattatggtagacaaagctcttccacttttagtgcatgaatttc 180
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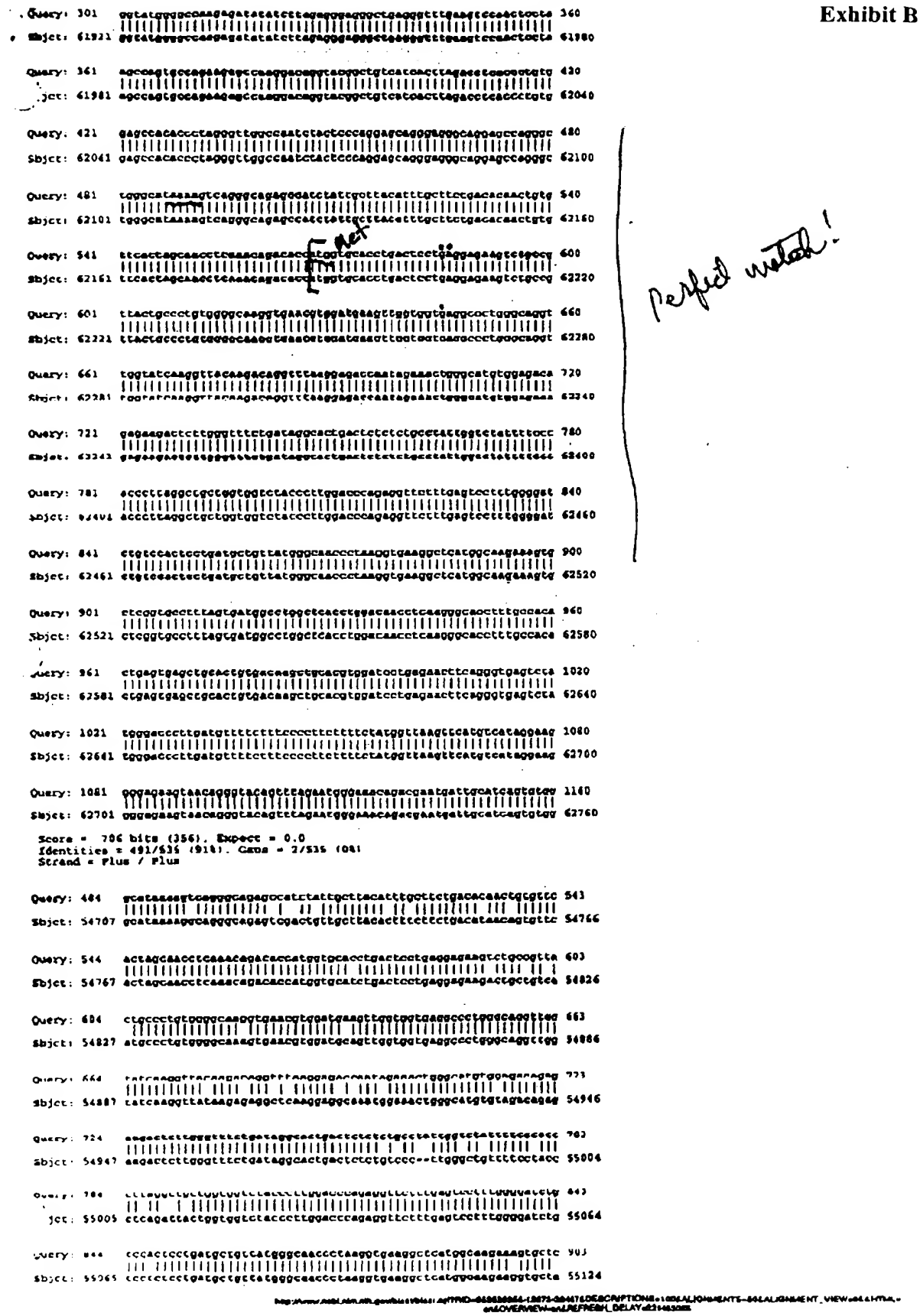
Exhibit B



ARDC-119
5' NCA ACT GGG ACC ATT G 3'
N = Cy5

ARDC-120
5' NTT CTC C(T/A)C AGG AGT C 3'
N = C6 Amino modifier

ARDC-121
5' NAG GGC CTC ACC ACC A 3'
N = C6 Amino modifier

ARDC-122
5' NAA CAC CAA AGA TGA T 3'
N = C6 Amino modifier

ARDC-123
5' NCC TTA CCT GGC AGT G 3'
N = C6 Amino modifier

ARDC-124
5' NCA ATG GTT CCA GTT G 3'
N = C6 Amino modifier

ARDC-109
5' NGG TAG TAA TGA GCG TGC AGC 3'
N = C6 Amino modifier

ARDC-110
5' NGA CTC CTG (A/T)GG AGA A 3'
N = Cy3

ARDC-111
5' NGA CTC CTA (A/T)GG AGA A 3'
N = Cy5

ARDC-112
5' NTG GTG GTG AGG CCC T 3'
N = Cy5

ARDC-113
5' NTG GTG GTA AGG CCC T 3'
N = Cy3

ARDC-114
5' NAT CAT CTT TGG TGT T 3'
N = Cy3

ARDC-115
5' NTA TCA TCG GTG TTT C 3'
N = Cy5

ARDC-116
5' NCA CTG CCA GGT AAG G 3'
N = Cy5

ARDC-117
5' NCA CTG CCG GGT AAG G 3'
N = Cy3

ARDC-118
5' NCA ACT GGA ACC ATT G 3'
N = Cy3

ARDC-100
5' NAA ACA GAC ACC ATG GTG CAC 3'
N = C6 Amino modifier

ARDC-101
5' NCC CAC AGG GCA GTA ACG GCA 3'
N = C6 Amino modifier

ARDC-102
5' NGC AAG GTG AAC GTG GAT GAA 3'
N = C6 Amino modifier

ARDC-103
5' NGT AAC CTT GAT ACC AAC CTG 3'
N = C6 Amino modifier

ARDC-104
5' NCT GGC ACC ATT AAA GAA AAT 3'
N = C6 Amino modifier

ARDC-105
5' NTT CTG TAT CTA TAT TCA TCA 3'
N = C6 Amino modifier

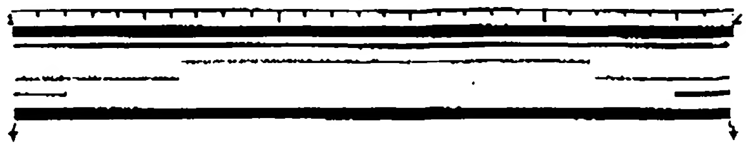
ARDC-106
5' NTG GGC TGT TCT AAC CCC CAC 3'
N = C6 Amino modifier

ARDC-107
5' NAA CCC ACT GGA GCC CCT GAC 3'
N = C6 Amino modifier

ARDC-108
5' NCC ACA GGA TCA GAG GCT GGG 3'
N = C6 Amino modifier

NCBI Nucleotide

Homo sapiens galactose-1-phosphate uridyl transferase (GALT) gene, exon 10, with an N314D mutation prevalent in Duarte galactosemia (M96264 bases 2981-3250)



Note: mutant shown here!

WT AAC=Asn
mutant GAC=Asp

```
         10         20         30         40         50         60
  1 AGGTGCTAAC CTCCATAACT GTAAAAGGGC TCTCTCTCCC CACTGTCTCT CTTCTTTCTG
                                                                     GALT
    primer_bind                             A
         70         80         90        100        110        120
 61 TCAGGGGCTC CCACAGGATC AGAGCCTGGC GCCAACTCCC ACCATTGGCA GCTCCACCCT
                                                                     GALT
            ARJC-108                                    ARDC-109
      A  P   T  C  S   E  A  G  A   N  H  D   H  H  Q   L  H  A  H
                                    ▲mutation
        130        140        150        160        170        180
121 CATTACTACC CTCCGCTCCT CCCCTCTGCC ACTGTCCGGA AATTCATGGT TGGCTACGAA
                                                                     GALT
      Y  Y  P   P  L  L   R  S  A  T   V  R  K   F  M  V   G  Y  E  M
        190        200        210        220        230        240
181 ATGCTTCCTC AGCCTCAGAG GGACCTCACC CCTCAGCAGG TCAGGACTCA GAACACTCTC
                                                                     GALT
      L  A  Q   A  Q  R   D  L  T  P   E  Q
        250        260        270
241 GCGTCTCCAC ACTCTCACAT GCAGTATGTG
                                    GALT
                                    primer_bind
```

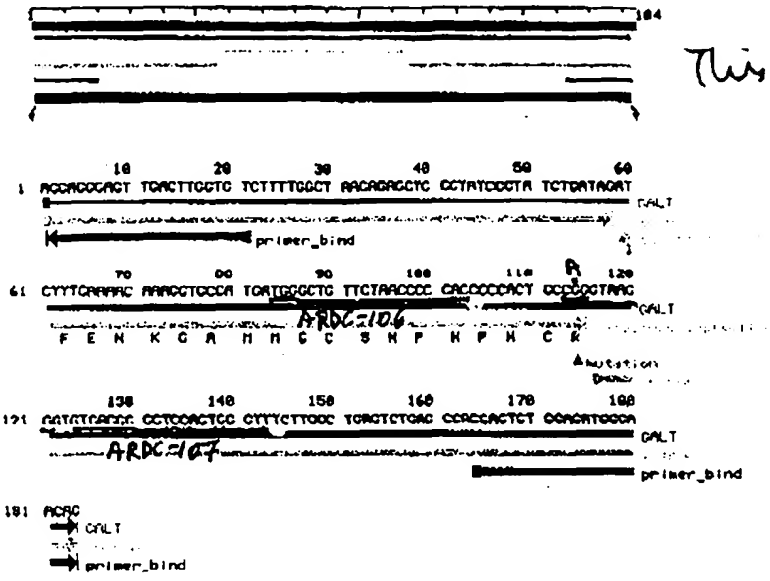
Go to : 1 Search for :

Comments and suggestions to: [info@ncbi.nlm.nih.gov]

Duarte allele: WT (a, Asn) N→D (mutant)
mutant (g, Asp) N314D

NCBI Nucleotide

Homo sapiens galactose-1-phosphate uridyl transferase (GALT) gene, exon 6, with a Q188R mutation prevalent in G/G Caucasian population causing reduced GALT activity (M96266 bases 1687-1870)



This is the mutant!

CAG = Gln(Q)
CGG = Arg(R)

Q188R

Go to: 1 Search for:

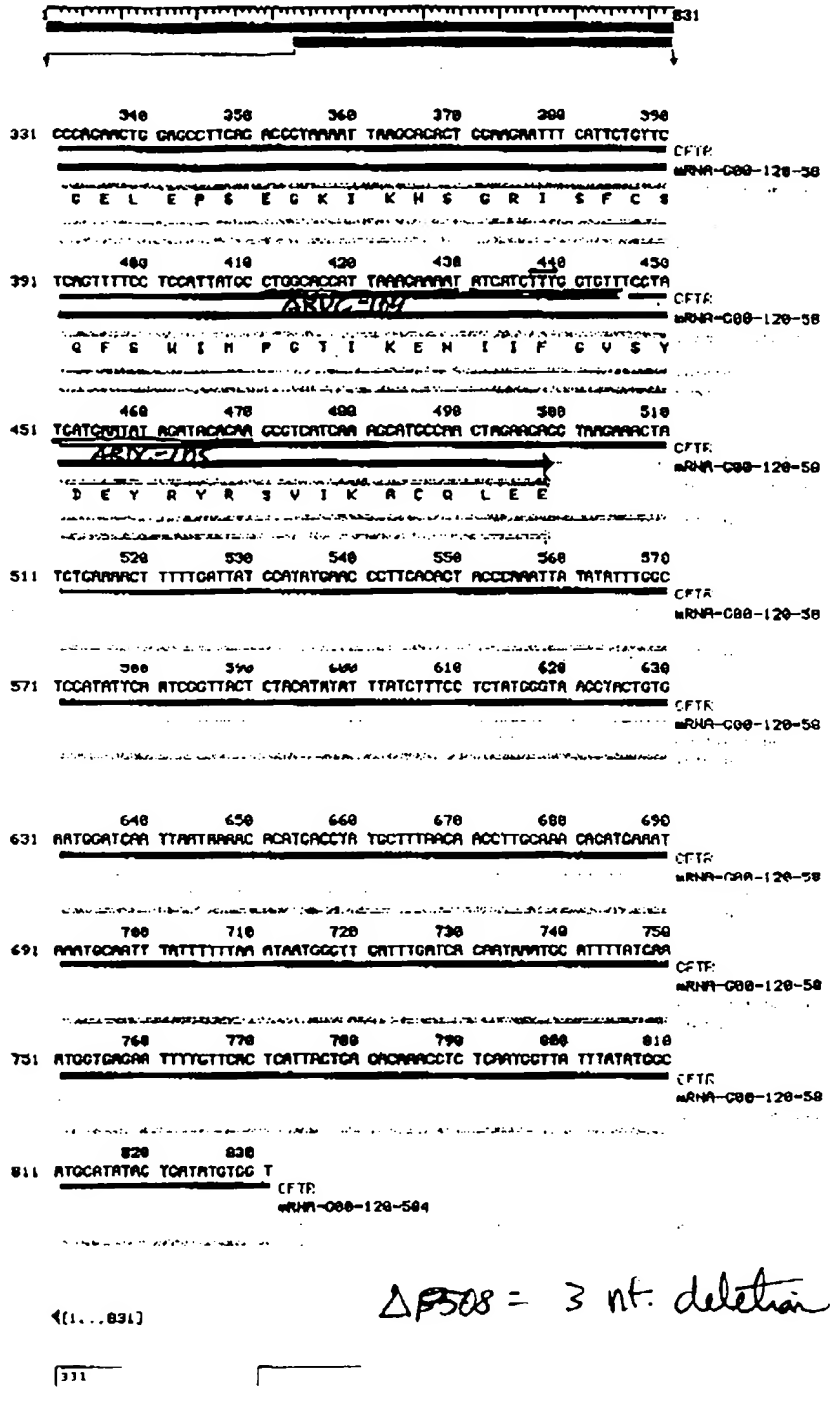
Comments and suggestions to: [info@ncbi.nlm.nih.gov]

WT(a, Gln)
Mutant(g, Arg)

a → g

NCBI Nucleotide

Human cystic fibrosis transmembrane conductance regulator (CFTR) gene, exon 10 (331..831)



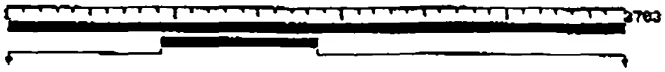
ΔFS
ΔTTT = ~~ΔF508~~
TTT = Phe(F)

ΔF508 = 3 nt. deletion of WT Sequence

Comments and suggestions to: (info@ncbi.nlm.nih.gov)

NCBI Nucleotide

Human beta-globin gene from a thalassemia patient, complete cds [921..1852]




```
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 921 CTATTTTTAC ACATAATTTA TTACCATCCA TGAGCAAATT AACAAAAACA ACAACAAATC
            1040       1060       1080       1100       1120       1140
 981 AATCCATATA TATGTATATC TATCTCTCTA CATATACACA TATATATATA TATTTTTTTT
            1160       1180       1200       1220       1240       1260
1041 CTTTTCTTAC CAGAAGCTTT TAATCCAAAT AAGGAGAACA TATCCTTAGA ACTCACCTAC
            1280       1300       1320       1340       1360       1380
1101 ACTTTTCATC CATTCTGTCC TCTAAGTATT TTCCATATTC TCCAGACCCA GCAAGACATC
            1400       1420       1440       1460       1480       1500
1161 CATCTACATA TCCCAAAGCT GAATTATCCT AGACAAAACT CTTCCACTTT TAGTCCATCA

            1520       1540       1560       1580       1600       1620
1221 ATTTCTTATT TGTGTAATAA CAAAATTGGG AAAACCATCT TCAATATGCT TACCAAGCTG
            1640       1660       1680       1700       1720       1740
1281 TCATTCCAAA TATTACCTAA ATACACTTCC AAACCACCAT GTTTTTACTA CCAATTTGTA
            1760       1780       1800       1820       1840       1860
1341 CTCATCGTAT GCCGCCAACA CATATATCTT ACACCCACCG CTCAGCGTTT CAAGTCCAAC
            1880       1900       1920       1940       1960       1980
1401 TCCTAAGCCA CTCCCAGAAC AGCCAACCAC ACCTACGGCT CTCATCACTT AGACCTCACC
            2000       2020       2040       2060       2080       2100
1461 CTCTCCAGCC ACACCCTACC CTTGCCCAAT CTACTCCCAC CAGCAGCGAC CCCACCACCC
                                    CAAT_signal

            2120       2140       2160       2180       2200       2220
1521 AGCCCTCCCC ATAAAAGTCA GCCCAGAGCC ATCTATTGCT TACATTTGCT TCTGACACAA
                 TATA_signal                          A T
            2240       2260       2280       2300       2320       2340
1581 CTGTGTTCAC TAGCAACCTC AAACAGACAC CATGCCTCCAC CTGACTCCTG AGGAGAAGTC
                                    M  V  H  L  T  P  E  E  K  S
            2360       2380       2400       2420       2440       2460
1641 TCCCCTTACT GCCCTGTGCC GCAAGGTGAA CGTGGATGAA GTTGGTGGTC AGGCCCTGGC
      A  V  Y  R  L  H  C  K  V  H  V  D  E  V  G  G  E  A  L  G
            2480       2500       2520       2540       2560       2580
1781 CAGCTTCCTA TCAAGCTTAC AAGACAGGTT TAAGGAGACC AATAGAAACT GGGCATGTGC
            2600       2620       2640       2660       2680       2700
1761 AGACAGAGAA GACTCTTCGG TTTCTGATAG GCACTGACTC TCTCTGCCTA TTGGTCTATT

            2720       2740       2760
1821 TTCCCAGGCT TACCCTGCTC GTGGTCTACC CT
                    L  L  V  V  Y  P
```

ARDC-100

ARDC-101

ARDC-102

ARDC-103

E+on1

G172A = C allele
A173T = S allele
G232A = E allele

GAG (Glu) → AAG (Lys)
GAG (Glu) → GTG (Val)
GAG (Glu) → AAG (Lys)

Use 6/3/98 Formula From Schena write.

$T_c = -682 \times (L^{-1}) + 97$

$T_m = T_c - 10°C$.

15-mer $-T_m = 42°C$.
13-mer $-T_m = 35°C$.
11-mer $-T_m = 25°C$.
9-mer $-T_m = 11°C$

10-mer $T_m = 19°C$.

Exhibit B

Neonatal Screening

Obtain 108 samples from Neo Gen
6 rows of 12 PCR tubes
Correspond to CF, B-globin and other loci and to Neo Gen samples A, B, C, D, E and F
50 ul PCR with 15 ul missing for agarose gels (gel bands look good)
Products look OK
All stored at –80C since arrival on

Remove from –80C, 72 samples and thaw
These correspond to 6 human loci, with quads of each locus and each genotype (wt, hetero and homo)

-Such that a given locus has 12 tubes

A. deltaF508WT, Heter, Homo
B. B-globin 172/173 S/S, A/S, S/C
C. B-globin 172/173 C/C, A/C, A/A
D. B-globin 232 E/E, A/E, A/A
E. GALT 314 WT, Hetero, Homo
F. GALT 188 WT, Hetero, Homo

Add 160 ml of binding buffer
Mix 10X
Add to 384-well filter plate

-Add so that each set of four occupies a quad of wells
-ie. The first set of four is in A1, A2, B1, B2 and so forth
-Leave the 10th set of wells (A9, A10, B9, B10) empty so that samples will fall on even rows when printing 30 x30 in triplicate. ie. Last set of three spots will be empty for the first row.

Add 3 times to filter entire 200 ul vol
Spin briefly between loadings, then 5 min to dry filter
Add 50 ul H2O, wait 2 min and elute by cent for 5 min
Allow 384-well plate to dry o/n under hood fan to dryness

After o/n drying under hood, the samples still contained ~20ul liquid
Dry in speedvac for 1.5 hrs at medium heat.
Add 5 ul H2O to each well and mix well
Add 5 ul of 2X MSS-1 to each well and mix
To new 384-well plate, transfer 3 ul of each sample.
Also, add 3 ul to 5 additional quads of wells for each of 5 control 15-mer oligos

-Oligos are A5, B5, C5, D5, E5 (ARDC120-124) from weboligos 3/21/00 plate stored at –20C
-Oligos are amino-mods at 100 uM concentration, diluted 1:1 with 2X MSS-1 for a final conc of 50 uM.

Spin plate 5 min at 500 x g to move samples to bottom of plate
Array onto 30 SuperAldehydes using triplicate spotting at 140 uM spacing and 30 x 30 config

-Note that final arrays should contain 4 identical subgrids with 2 complete rows and the third containing 12 spots. The final 3 spots in row 1 should be 1X MSS-1

Printing looks good!
Store arrays in a substrate box for processing.

After o/n drying, label 2 arrays barcoded # 105034 and 105035
Demarcate array with diamond pencil on underside
Process as per published protocols
Soak 2X in 0.2% SDS
Soak 2X in dH2O
Treat for 2 min at 95C in dH2O
Spin dry 1 min

Treat for 5 min in sodium borohydride (1.0 g in 400 ml dH2O)
Rinse 3X in 0.2% SDS
Rinse 1X in dH2O
Treat 2 sec at 95C in dH2O
Spin dry 1 min

Hybridization:
Set array in hyb cassette
Add 10 ul of dH2O
Prepare 2 cover slips 22 mm x 22 mm
Lay cover slip on top of array
Add 10 ul of 2-color fluorescent probes
-Probe are mixtures of 10 fluorescent oligos (5 Cy3 and 5 Cy5)
Oligos are from weboligos 3/21/00 plate
-Cy3 are A1, B1, C1, D1 and E1
-Cy5 are A3, B3, C3, D3 and E3
-All 10 are in a 10 uM mixture stored at –20C
Probe 1: 10 oligos at 1 uM each final conc in 1X UniHyb
Probe 2: 10 oligos at 1 uM each final conc in 5X SSC + 0.2% SDS
Add probe 1 to array 105034 and probe 2 to array 105035
Hyb at 42C for 1.5 hrs
Wash 2X in 2X SSC + 0.2% SDS and 1X in 2X SSC for 5 min each
Spin dry 1 min
Scan at 100% PMT and 100% laser

Results:
Signals are rather weak but background is very low
Looks like the experiment is working!
See scans ms000420a-d
a-Cy3 with array 105034
b-Cy5 with array 105034
c-Cy3 with array 105035
d-Cy5 with array 105035

Second chip (SSC and SDS) slightly brighter signal. Quant data

Processing:
Process chips and compare direct labeling vs. NEN TSA on neonatal chips
Obtain 4 chips from rt drawer. Chips were made on SuperAldehyde on 4/19/00
Bar code as 105227-105230
Mark array with diamond pencil
Wash 2 x 2 min in 0.2% SDS and 2 x 2 min in dH2O.
Denature 2 min at 95-100C in dH2O.
Reduce in NaBH4 for 5 min at rt [320 ml dH2O + 1.2 g NaBH4 + 120 ml 100% ethanol]
Wash 2 x 2 min in 0.2% SDS, 2 x 2 min in dH2O. Spin dry.
Use for Hyb.

Hybs:

Exhibit B

Probes-

1. The Cy3/Cy5 mixture prepared on 4/20/00 and stored frozen at –20C. Mixture of 5 Cy3 oligos and 5 Cy5 oligos end-labeled corresponding to 1A-E and 3A-E from 3/24/00 weboligos source. All at 10 uM each. Make hyb mixture by mixing:
   3 ul of 10 uM oligo mix
   7.5 ul of 20X SSC
   6 ul of 1% SDS
   13.5 ul of dH20
   30 ul total volume.

Heat for 1 min at 65C
Spin for 1 min
Hyb to 105227 and 105228 under 22 mm x 22 mm cover slip, using 10 ul hyb solution per chip.

2. The biotin/DNA mixture prepared fresh on 6/7/00 and stored frozen at –20C after use. Mixture of 5 biotin oligos and 5 DNP oligos end-labeled corresponding to 1A-E and 7A-B from 5/24/00 weboligos source. All at 10 uM each. Make hyb mixture by mixing:
   3 ul of 10 uM oligo mix
   7.5 ul of 20X SSC
   6 ul of 1% SDS
   13.5 ul of dH20
   30 ul total volume.

Heat for 1 min at 65C
Spin for 1 min
Hyb to 105229 and 105230 under 22 mm x 22 mm cover slip, using 10 ul hyb solution per chip and 10 ul dH2O for humidification.
Hyb 4.5 hrs at 42C
Wash 2 x 5 min in 2X SSC + 0.2% SDS and 1 x 5 min in 2X SSC
Spin dry.
Scan chips 105227 and 105228 at 100% PMT and 100% laser with ScanArray 3000.

Exhibit B

		Cy3 raw	Cy3 Ave.	Cy3 Ave-Backgr.	Cy5 raw	Cy5 Ave.
Spot 28	1X MSS-1	2779			949	
Spot 29	"	3063	2964	0	1106	1123
Spot 30	"	3021			1313	
Spot 31	B-globin 232E/E	4986			1396	
Spot 32	B-globin 232E/E	5246	5358	2404	1395	1606
Spot 33	B-globin 232E/E	5841			2028	
Spot 34	B-globin 232A/E	3918			1831	
Spot 35	B-globin 232A/E	3706	3831	877	1429	1566
Spot 36	B-globin 232A/E	3868			1439	
Spot 37	B-globin 232A/A	3483			2871	
Spot 38	B-globin 232A/A	3126	3319	365	3133	2715
Spot 39	B-globin 232A/A	3347			2141	
		46384			21031	

Quantitation of two color genotyping on Neonatal chips printed on 4/19/00
Mixture of 10 fluorescent oligos to 5 loci in Cy3 and Cy5
Hyb buffer was 5X SSC + 0.2% SDS for 1.5 hrs at 42C
Probe solution was 1 uM each oligo
Washes were RT in 2X SSC + 0.2% SDS twice and once in 2X SSC
Scans were on GSIL 3000 at 100% laser and 100% PMT

Exhibit B

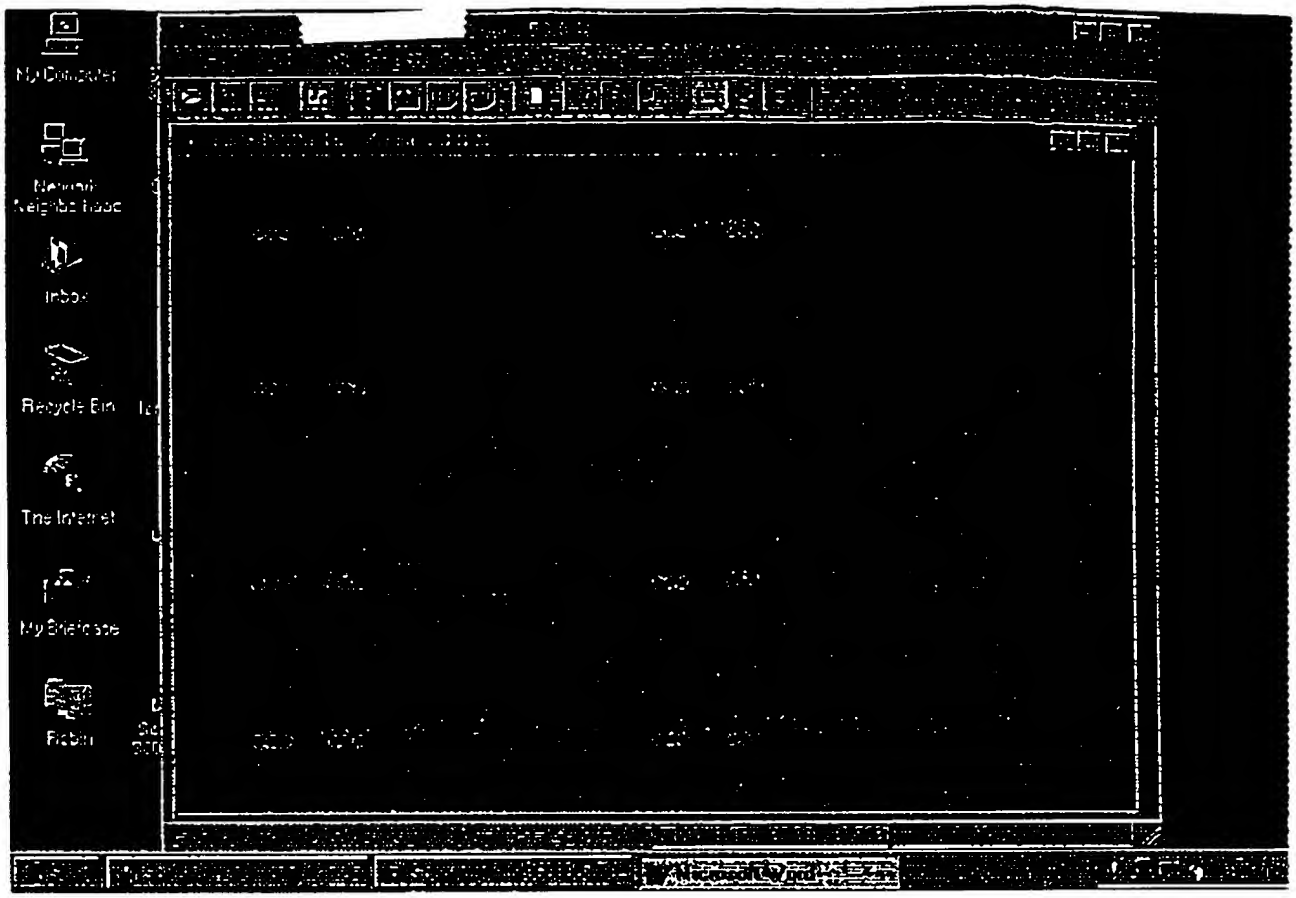

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Exhibit B

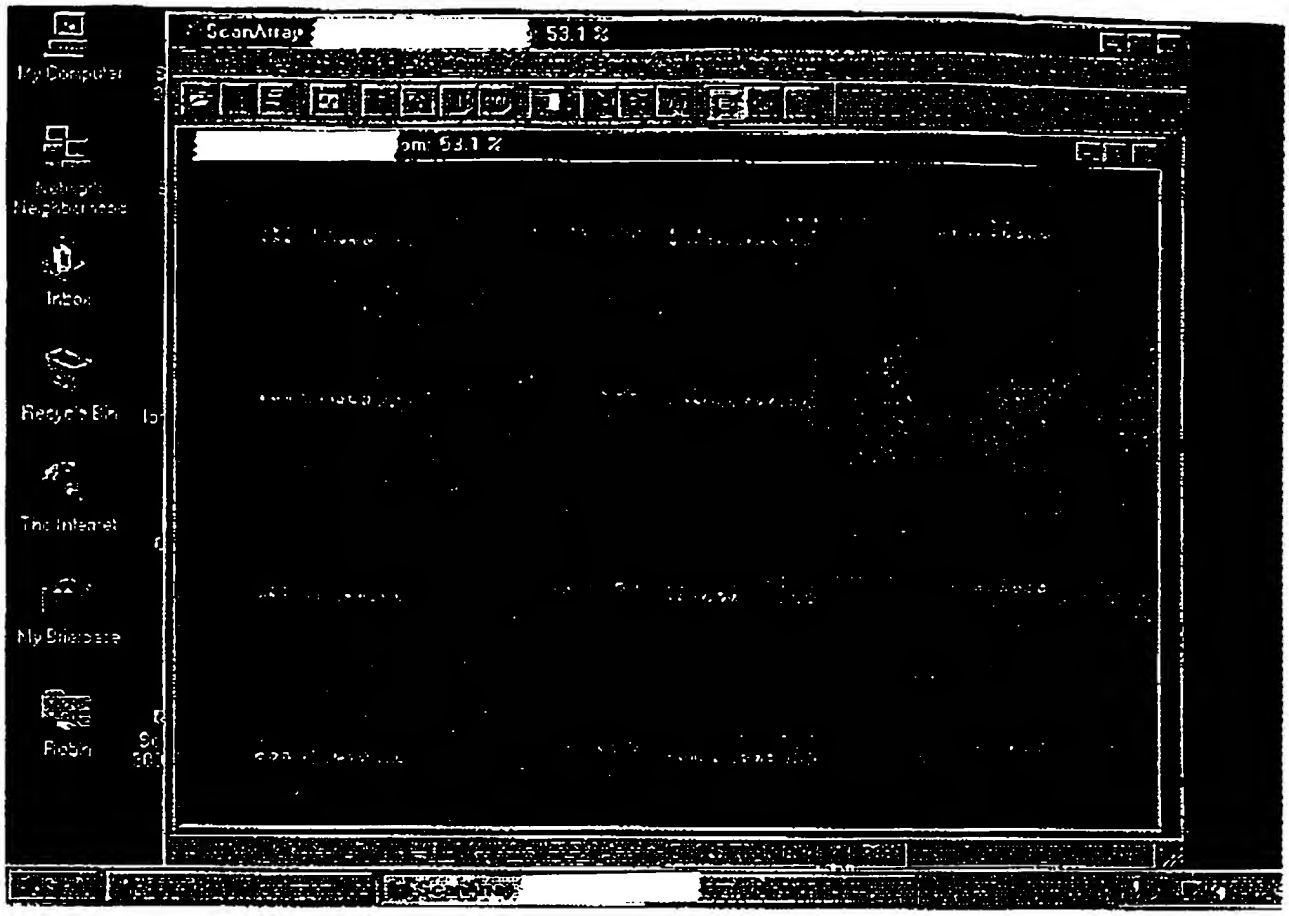

ScanArray
53.1 %
My Computer
Inbox
Recycle Bin
The Internet
My Briefcase
Robin

Exhibit B

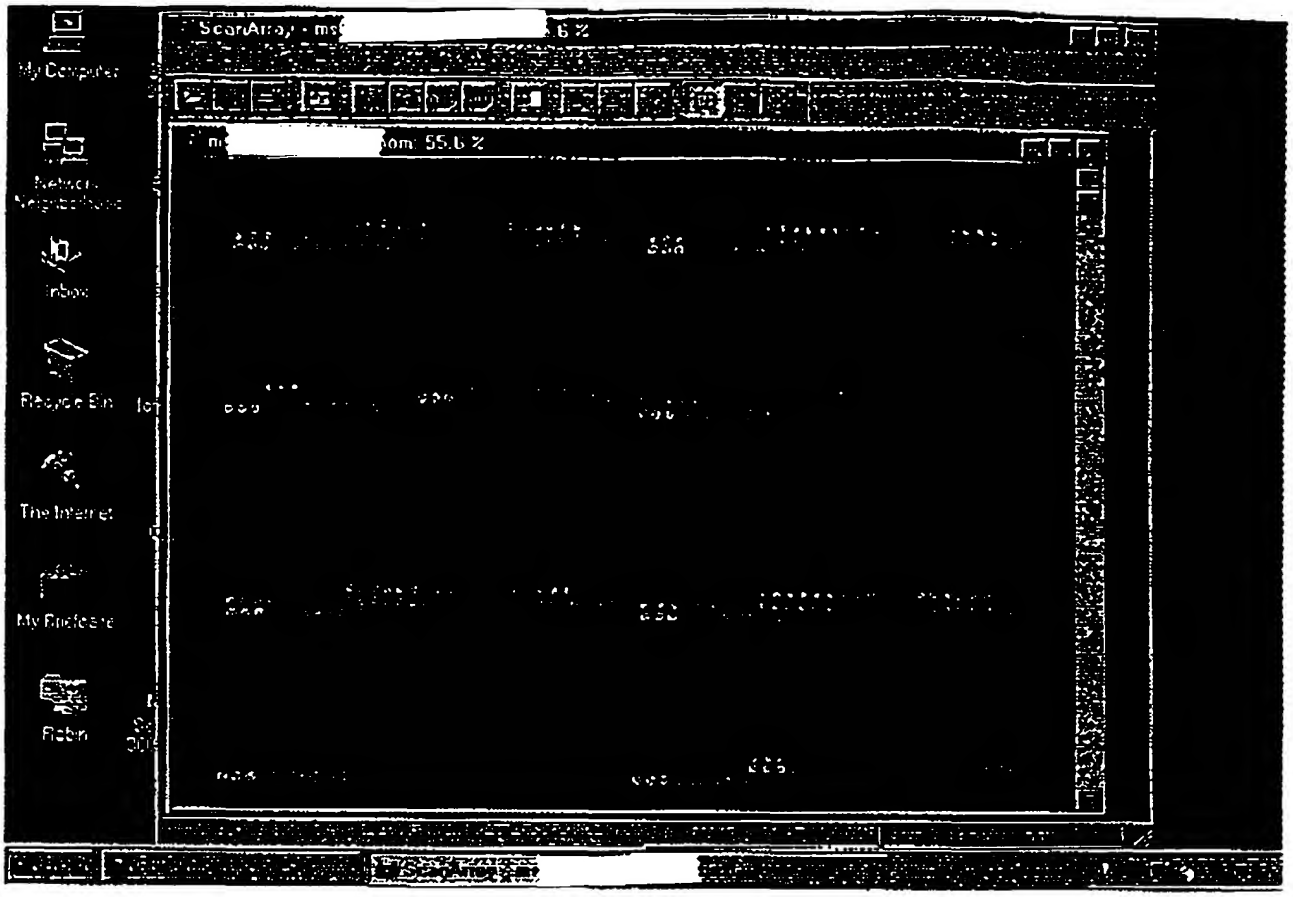


Exhibit B

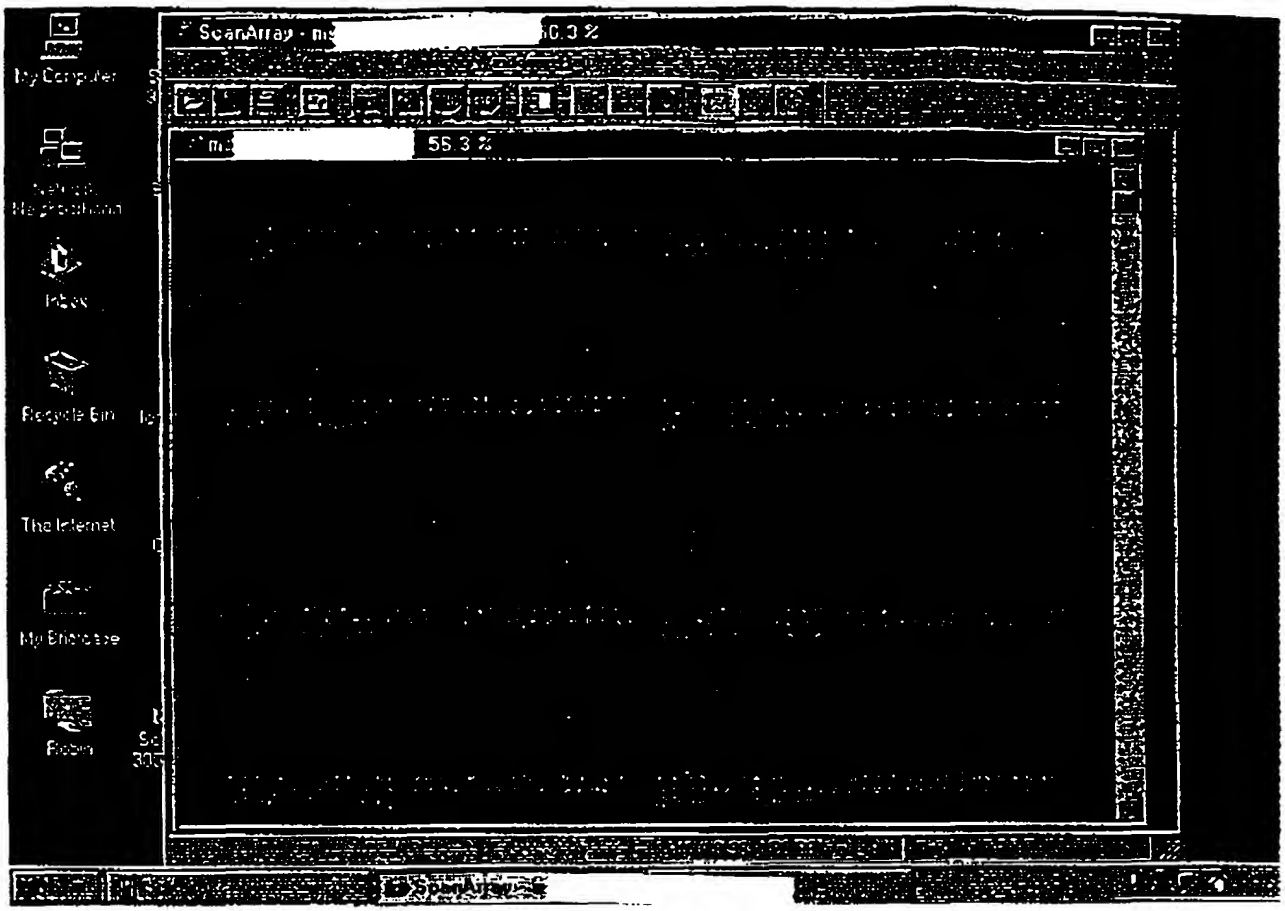


Exhibit B

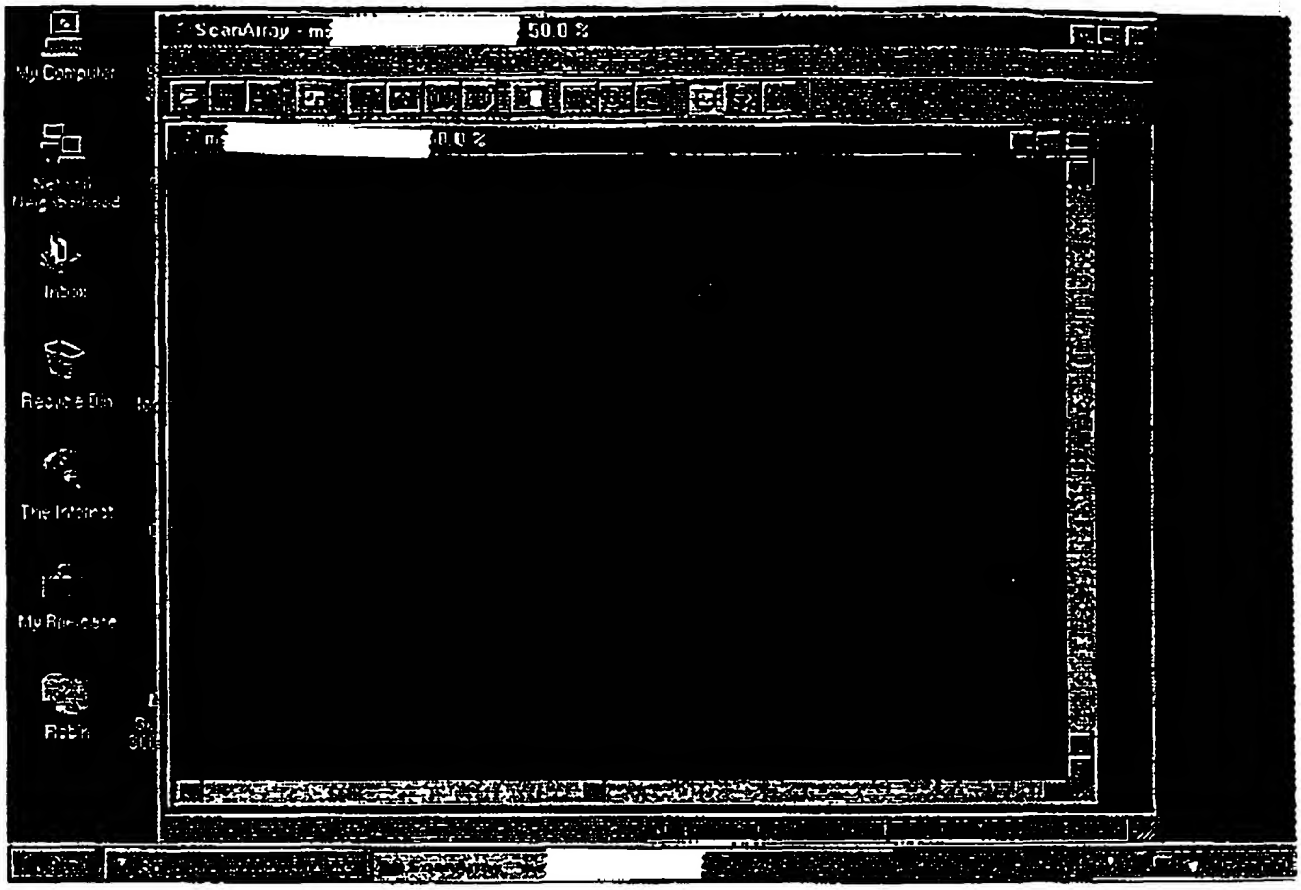

ScanArray - m
50.0 %
My Computer
Inbox
Recycle Bin
The Internet
My Briefcase
Robin

Exhibit B

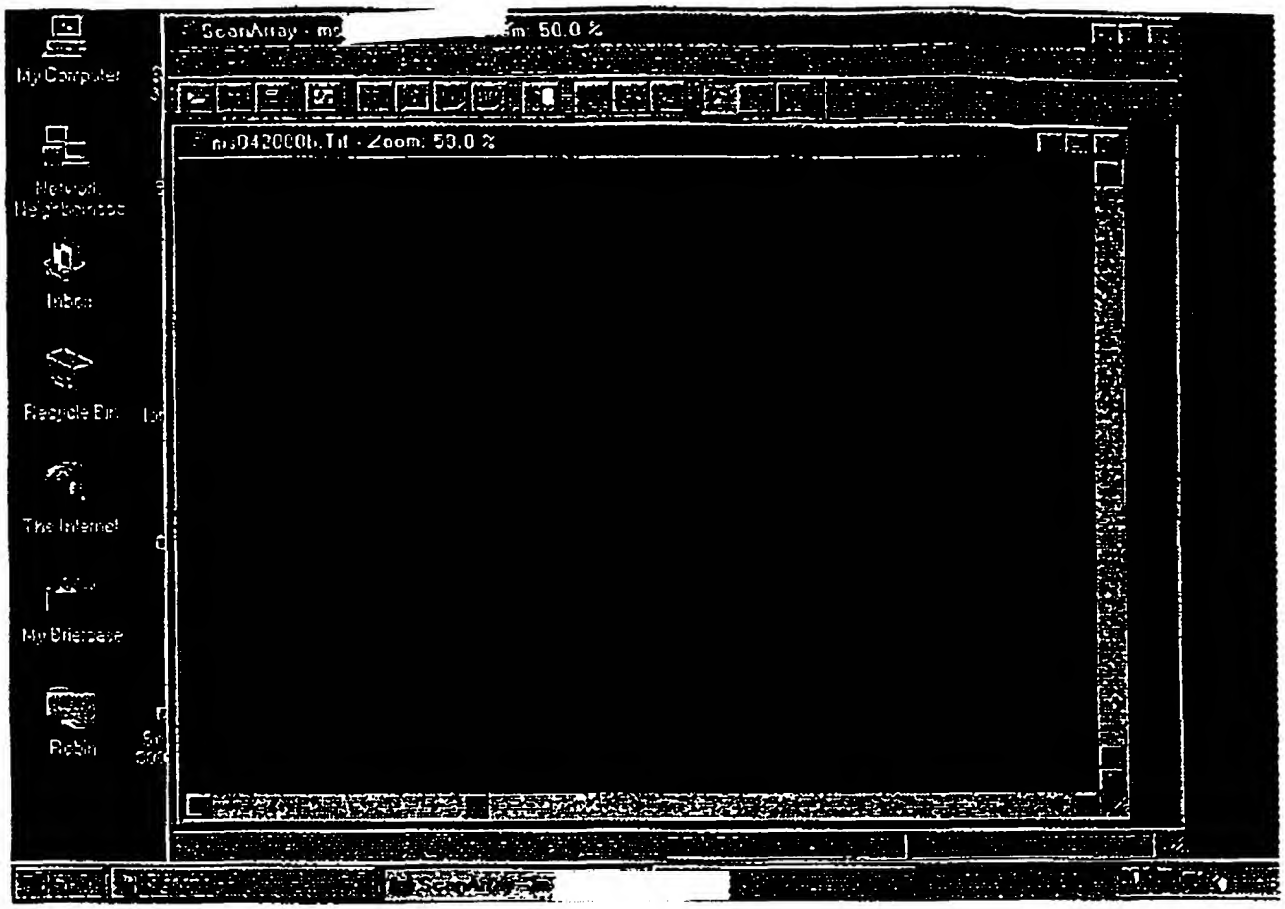

My Computer
Inbox
The Internet
My Briefcase
Recycle Bin
Zoom: 50.0 %

Exhibit B

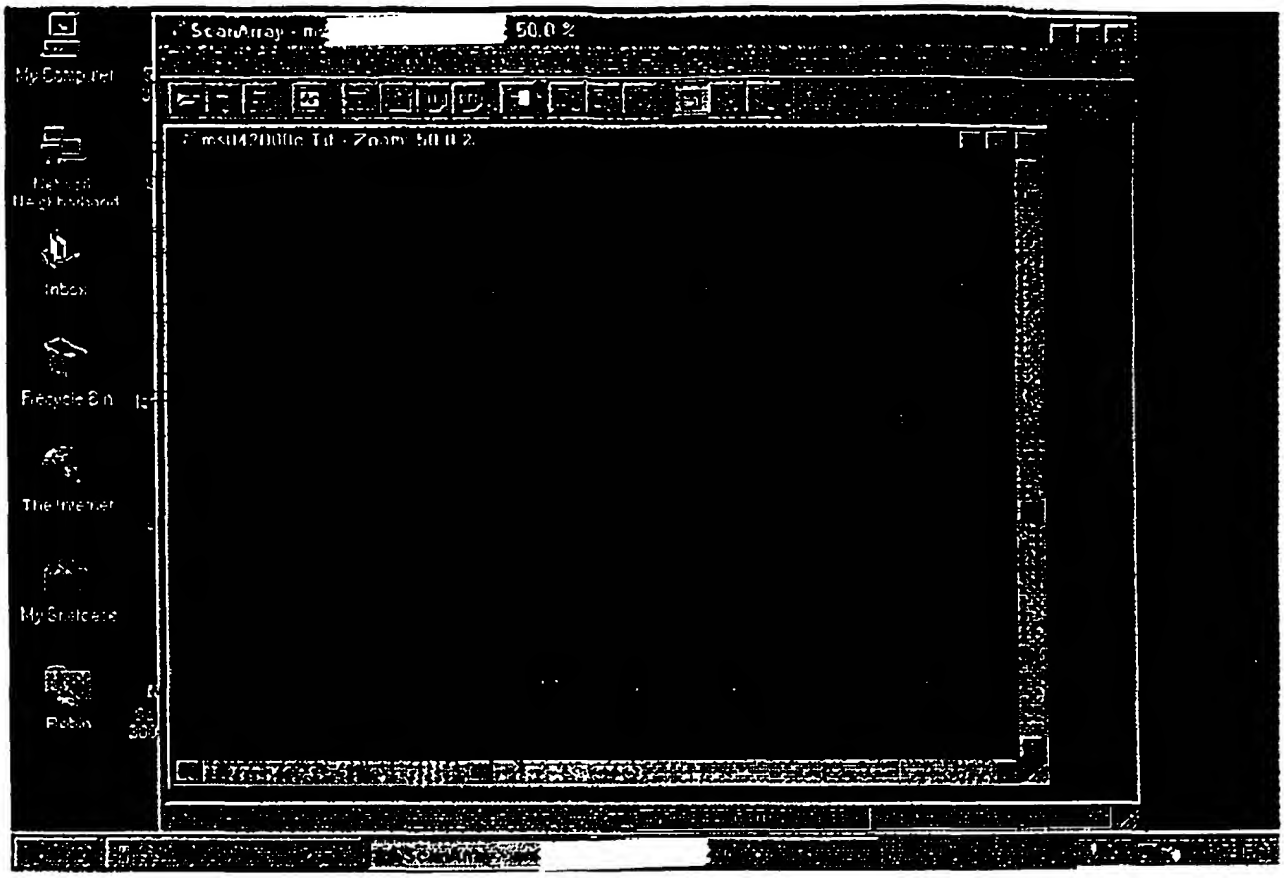
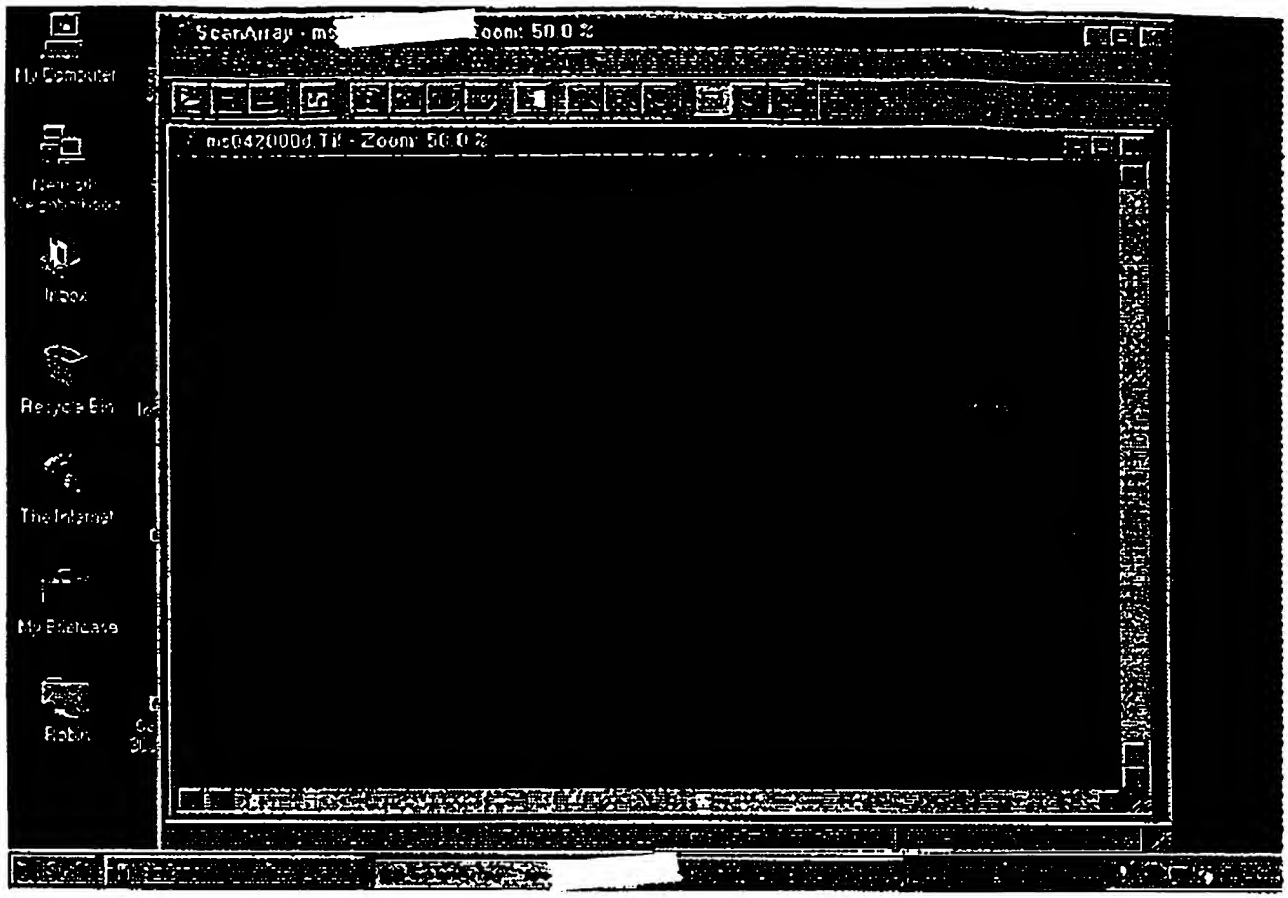


Exhibit B


My Computer
Inbox
Recycle Bin
The Internet
My Briefcase
Robin
ms0420000d.TIF - Zoom: 50.0 %